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Charophytes collapse beyond a critical warming and brownification threshold in shallow lake systems

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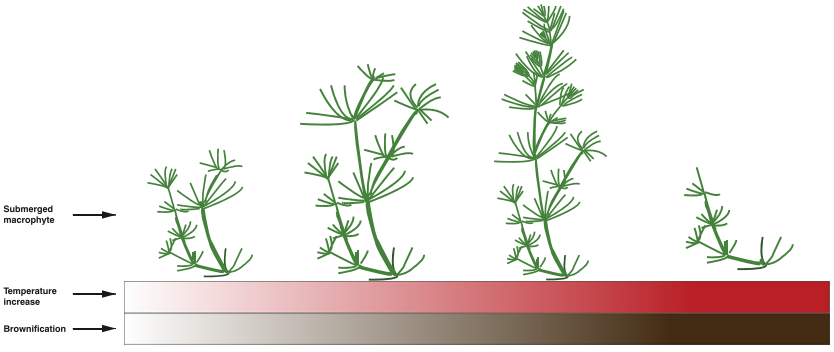
HIGHLIGHTS

- *Chara vulgaris* had higher growth at +2 °C and +100% brownification.
- Growth of *C. vulgaris* declined at +4 °C and 200% brownification.
- Studied charophyte growth will decline as warming and brownification increased.

GRAPHICAL ABSTRACT



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ABSTRACT

Charophytes play a critical role for the functioning of shallow lake ecosystems. Although growth of charophytes can be limited by many factors, such as temperature, nutrients and light availability, our understanding about concomitant effects of climate warming and other large-scale environmental perturbations, e.g. increases in humic matter content ('brownification') is still limited. Here we conducted an outdoor mesocosm experiment during 71 days with a common charophyte species, *Chara vulgaris*, along an increasing gradient of temperature and brownification. We hypothesized the growth of *C. vulgaris* to increase with temperature, but to level off along the combined temperature and brownification gradient when reaching a critical threshold for light limitation via brownification. We show that *C. vulgaris* increases the relative growth rate (RGR), main and total shoot elongation, as well as number of lateral shoots when temperature and brownification increased by +2 °C and + 100%, respectively above today's levels. However, the RGR, shoot elongation and number of lateral shoots declined at further increment of temperature and brownification. Macrophyte weight-length ratio decreased with increased temperature and brownification, indicating that *C. vulgaris* allocate more resources or energy for shoot elongation instead of biomass increase at warmer temperatures and higher

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Morphology
Submerged macrophyte

brownification. Our study shows that *C. vulgaris* will initially benefit from warming and brownification but will then decline as a future scenario of increased warming and brownification reaches a certain threshold level, in case of our experiment at +4 °C and a 2-fold increase in brownification above today's levels.

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1. Introduction

Submerged macrophytes including charophytes (macroalgae) play an important role for the ecosystem functioning in shallow freshwater ecosystems (Blindow et al., 2014; Burks et al., 2006; Meerhoff et al., 2003). Charophytes improve nutrient retention and water clarity, reduce the re-suspension of sediment particles, provide refuges for small fishes and zooplankton and affect competitive outcomes with other primary producers, e.g., phytoplankton and periphyton (O'Hare et al., 2018; Blindow et al., 2014; Rojo et al., 2013; van den Berg et al., 1998). The growth of charophytes can be limited by many environmental factors, e.g., light availability, salinity, nutrients and temperature (Puche et al., 2018; Rojo et al., 2017; Sanjuan et al., 2017). Charophytes and other submerged macrophytes generally show higher growth rates at warmer temperatures (Li et al., 2017; Joye and Rey-Boissezon, 2015; Mckee et al., 2002), but they often decline with increasing shading or decreasing water transparency (Hilt et al., 2018; Hidding et al., 2016). However, at present little is known about the concomitant effects on charophytes of the global warming and other large-scale environmental perturbations, which are of considerable concern (Rojo et al., 2017; Pelechata et al., 2015).

According to the IPCC (2013), the global average temperature will increase between 0.3 °C to 6.4 °C by the end of this century. This may have profound effects on biodiversity and ecosystem functioning of fresh waters, particularly for lakes (Moss et al., 2003), as well as the growth of charophytes (Puche et al., 2018). Charophytes might benefit from warming (Joye and Rey-Boissezon, 2015) due to higher photosynthetic rates, light use efficiency and growth (Rojo et al., 2017; Rooney and Kalff, 2000; Barko and Smart, 1981). Besides global warming, other large-scale environmental changes are expected to occur simultaneously in the future, for example, increases in humic substances reaching freshwaters from the surrounding land have dramatically increased in northern Europe and North America in last decades, a phenomenon known as 'brownification' (Monteith et al., 2007). Transportation of humic substances from terrestrial to aquatic systems due to increased precipitation will further intensify the brownification of freshwater systems in the future (Weyhenmeyer et al., 2016). Brownification can have both positive and negative effects on charophytes; for example, photodegradation of DOC (dissolve organic carbon) can increase CO_2 concentrations in lakes and thereby enhance photosynthesis and macrophyte growth (Reitsema et al., 2018). In addition, DOC attenuates UV-B radiation, which is harmful for macrophyte growth (Reitsema et al., 2018). On the other hand, brownification reduces the depth of the euphotic zone in lakes, and this will affect primary producers, such as phytoplankton and macrophytes, through light attenuation and thereby limiting photosynthesis (Mormul et al., 2012; Ask et al., 2009).

A major endeavor of the scientific community and water managers today is to reveal patterns of change along gradients of multiple environmental perturbations and so to identify abrupt biological responses of ecosystem functioning and biodiversity (Burkett et al., 2005). Future projections of climate warming and brownification trends show large spatial and temporal variation across the globe (Weyhenmeyer et al., 2016; IPCC, 2013; Easterling et al., 2000). Such spatio-temporal variation will expose charophytes in shallow lakes to strong gradients in temperature and brownification, even over a single growing season. Recent studies have shown strong effects of warming and brownification on the growth and biodiversity of other primary producers in lakes, either as combined pressures or in isolation (Li et al., 2017; Urrutia-Cordero et al., 2017; Hansson et al., 2013), yet our understanding on the responses of charophytes to gradients of such environmental perturbations remains elusive.

In this context, rather than investigating growth responses by charophytes to individual environmental pressures (warming or brownification in isolation), which are important for the mechanistic understanding of stressor-effects, but have commonly been assessed with either macrophytes or other primary producers (Li et al., 2017; Urrutia-Cordero et al., 2016b; Hansson et al., 2013; Mormul et al., 2012; Moss et al., 2003), here we employed a mesocosm experiment with a gradient design. This gradient consisted of six combined levels of increasing temperature and brownification in concert, as projected by future climate models (Weyhenmeyer et al., 2016; IPCC, 2013), and which enabled us to identify threshold levels after which charophyte growth was critically affected. We used *Chara vulgaris* as model organisms because charophytes are widely distributed and often dominate the primary producer communities of shallow temperate lakes (Schubert et al., 2018; Rey-Boissezon and Joye, 2015), where they strongly contribute to the stability of clear water states through positive feedback mechanisms (see Hilt et al., 2018; Schubert et al., 2018; Hidding et al., 2016). Hence, charophytes are often critical for avoiding regime shifts from clear water states to unhealthy, turbid states with frequent occurrence of toxic algal blooms (Urrutia-Cordero et al., 2016a; Urrutia-Cordero et al., 2015; Ekvall et al., 2014). We hypothesized that at the lower end of the gradient both brownification and temperature will have positive effects on the growth *C. vulgaris* due to faster metabolism of macrophytes at elevated temperatures (sensu Barko and Smart, 1981), as well as increased photosynthesis due to increased CO_2 released from degradation of DOC (sensu Salonen and Vähätalo, 1994). However, we also predicted the growth of *C. vulgaris* to level off along the temperature and brownification gradient when reaching a certain threshold for light limitation via brownification. Identifying the existence of such a threshold, and its approximate position along the gradient of warming and brownification, may be of considerable importance for managers and decision makers in preparation for large-scale changes in ecosystem function and services, as brownification and warming simultaneously increase in many freshwater environments.

2. Material and methods

2.1. Outdoor mesocosms

The outdoor mesocosm experiment consisted of 24 insulated cylindrical polyethylene containers (inner diameter 0.7 m, depth 1 m, volume 400 L) subject to natural light conditions. The mesocosms were randomly placed in a block design, with six mesocosms in each row and a total four rows, at the Lund University's experimental facilities (55°42′N, 13°12′E). The average daily total solar radiation for this area was 332 W m^{-2} (minimum: 58 Wm^{-2} and maximum: 498 Wm^{-2}) during the experimental period (Lund University weather station, SMHI, 2013). However, regular photosynthetic active radiation (PAR) measurements could not be made in mesocosms since the sun is never in zenith at the latitudes of Sweden and thereby the mesocosm walls will shade the incoming light in different mesocosms. Therefore, we calculated diffuse attenuation coefficient (K_d; cm^{-1}) for PAR based on Morris et al. (1995) by using the equation $K_{d\ PAR} = 1.30\ [a_{420}] + 0.12$ where, a_{420} = absorbance value at 420 nm. Our treatments

generated constantly different diffuse light absorptions (K_d) through the water column (see Fig. S1for diffuse absorption coefficient in control and particular treatments).

2.2. Temperature treatment

Each mesocosm was filled with 400 L of unfiltered lake water collected from the shallow eutrophic Lake Krankesjön (55°42′N, 13°27′E) located in southern Sweden (see Table S1 for water chemistry data of Lake Krankesjön). The water temperature in the mesocosms was controlled by a computerized system (for technical details, see Hansson et al., 2013) consisting of real-time temperature sensors and aquarium heaters (Jäger 150 W), which controlled the temperature in heated mesocosms in relation to ambient (control) conditions (Fig. 1). Each mesocosm received a gentle air flow to avoid temperature differences and water column stratification (Fig. 1). The daily average temperature in the control mesocosms was 19.20 °C (minimum: 15.48 °C, maximum: 23.88 °C) during the whole experimental period (see Fig. S2a). The mesocosms mimicked shallow lake ecosystems and therefore did not take into account the thermal stratification processes occurring in deeper lakes (Urrutia-Cordero et al., 2017).

2.3. Brownification treatment

Brownification, the increment of yellow-brown color of lake and stream water, strongly absorbs solar radiation in the short wavelength part of the visible spectrum (Graneli, 2012). Traditionally, brownification has been measured using a platinum salt solution as reference, which has today been substituted by measurements of absorbance at wavelengths around 400 nm (Graneli, 2012). We applied six treatments combining warming and brownification along a gradient: i) control treatment: no manipulation of temperature and brownification (or water color) of lake water, ii) treatment 1: +1 °C increase in temperature and 0.5-fold (50%) increase in brownification compared to control, iii) treatment 2: +2 °C increase in temperature and 1-fold (+100%) increase in brownification compared to control, iv) treatment 3: +3 °C increase in temperature and 1.5-fold (+150%) increase in brownification compared to control, v) treatment 4: +4 °C increase in temperature and 2-fold (+200%) increase in brownification compared to control and. vi) treatment 5: +5 °C increase in temperature and 2.5-fold (+250%) increase in brownification compared to control. All the treatments were replicated four times. To increase the brownification in the water along the treatment gradient (Fig. 1), we added once a week commercially available humic and fulvic acids (HuminFeed®, Humintech, Germany; see Urrutia-Cordero et al., 2016a, 2016b, 2017). The experimental treatments were manipulated using water color based on absorbance measurements at 420 nm as a proxy for brownification (Urrutia-Cordero et al., 2017; Weyhenmeyer et al., 2016). The increase in absorbance was maintained every week by adding humic substances from a stock solution in distilled water (1 g L^{-1}). No differences in pH or nutrients were recorded among treatments due to addition of humic substances (Fig. S2c and S3). The average pH in the controls was 8.93 (minimum: 7.42; maximum: 9.86) during the whole experimental period (for details see Fig. S2c). Distilled water was added every week to each mesocosm in order to compensate for evaporation and 1 mL of commercially available plant nutrients (Blomstra växtnäring, Cederroth, Upplands Visby, Sweden; 50.1 g L^{-1} total nitrogen and 10.0 g L^{-1} total phosphorus) was added every second week to maintain similar nutrient levels in all mesocosms (see Fig. S3 for total phosphorus and total N concentration in controls and particular treatments).

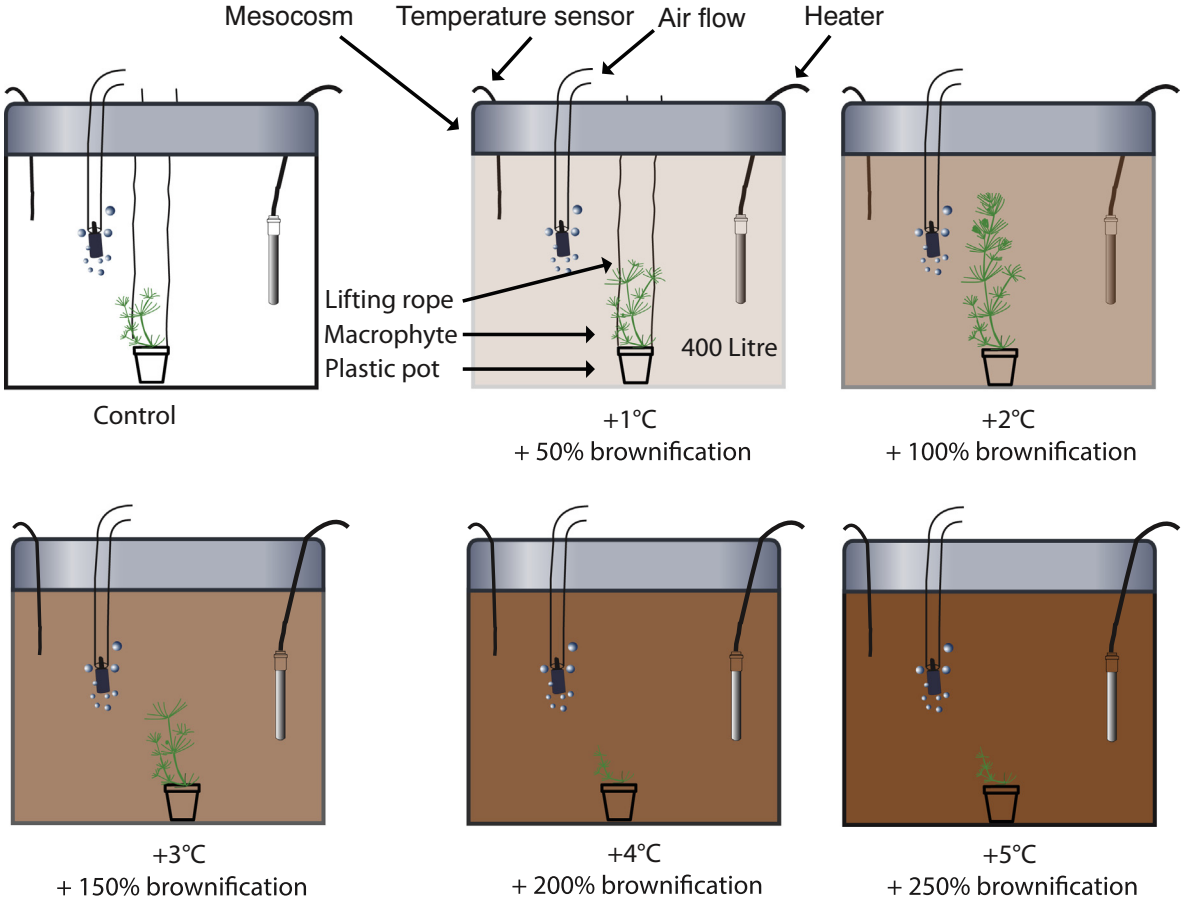

Fig. 1. Schematic diagram of experimental growth of submerged macrophyte, *Chara vulgaris*, along an increasing gradient of temperature and brownification.

Our experimental manipulations were based on temperature projections by climate models (IPCC, 2013) and brownification scenarios for southern Swedish lakes (Hansson et al., 2013) mimicking future projected increases in brownification for Swedish lakes during the coming 50–75 years (Weyhenmeyer et al., 2016). Temporal variation in temperature, absorbance and pH during the experimental period are given in the supplementary information, Fig. S2.

2.4. Macrophyte cultivation

Healthy and clean shoots of *C. vulgaris* were collected from lake Krankesjön and one 10 cm apical shoot (without any lateral shoots) was planted into small plastic pots filled with lake sediment and placed on a tray in each mesocosm. We used ropes to lift and lower the trays with pots for gentle and non-destructive sampling (Fig. 1). The macrophyte growth experiment ran between 13th May to 22nd July 2013, i.e. for a total of 71 days. Once a week we lifted the tray and counted the number of lateral shoots and measured the length of the main shoot and lateral shoots in each treatment. At the end of the experiment all the macrophytes were harvested, main shoot and total shoot length (including length of main shoot and lateral shoots) were recorded and plant dry weight was measured to the precision of four decimals on a Sartorius Analytic A210P balance after drying at 60 °C for 48 h (Termaks drying oven 8000 series). We measured relative growth rate (RGR) of *C. vulgaris* by using the formula, $RGR = (\ln W_2 - \ln W_1)/\Delta t$ where, W_1 = initial dry weight, W_2 = end dry weight, Δt = total number of days. For measuring initial dry weight of *C. vulgaris*, we used ten individual apical shoots similar to those used in mesocosm experiment (10 cm long each, without any lateral shoots). The shoots were dried at 60 °C for 48 h and weighted to the precision of four decimals as described above. We also measured the ratio of plant dry weight and total length at the end of the experiment according to Mormul et al. (2012).

3. Statistical analyses

We used one-way ANOVA to check for treatment effects on a) the RGR, b) the total increase of main shoot and total shoot lengths and c) number of lateral shoots at the end of experiment. A mixed-model ANOVA was used to check the effect of treatments on the increment rate of total shoot lengths at three sampling periods (i.e. May, June and July) where sampling time and treatments were considered as fixed factors and individual mesocosms as a random factor. Data of the ratio between macrophyte dry weight and length were log transformed prior to analysis in order to meet the assumptions of parametric tests. Tukey's pairwise comparison was used to test the significance of differences between the treatments. Data from treatment 3 (+3 °C and +150% brownification) was excluded from all statistical analyses since 3 out of 4 replicates of *C. vulgaris* died in this treatment after three weeks.

4. Results

The relative growth rate (RGR) of *C. vulgaris* significantly differed (One-way ANOVA, $F_{4, 11} = 6.45$, $p < 0.05$) between the treatments. In general, *C. vulgaris* showed higher RGR at treatment 2 (+2 °C and + 100% brownification) while RGR at treatment 4 (+4 °C and 200% brownification) was significantly lower than treatment 2 and ambient conditions (control) (Fig. 2A, Tukey's pairwise comparison, $p < 0.05$). The difference in RGR between treatment 2 and treatment 5 (+5 °C and 250% brownification) was marginally significant (Fig. 2A, Tukey's pairwise comparison, $p = 0.06$). Although we did not find any significant differences (One-way ANOVA, $p = 0.056$) between the treatments on total increment of main shoot length, there was a trend towards increased main shoot length with increasing temperature and brownification until treatment 2 along the gradient and then main shoot length declined with further brownification in treatment 4 and 5 (Fig. 2B). A similar trend was also observed for increment of total shoot lengths where treatment 2 showed significantly higher increment compared to treatment 4, treatment 5 and ambient conditions (Fig. 2C, one-way ANOVA, $F_{4, 11} = 7.33$, $p < 0.05$, Tukey's pairwise comparison). We found significant differences between the treatments on total number of lateral shoots at the end of the experiment (Fig. 2D, one-way ANOVA, $F_{4, 11} = 5.79$, $p < 0.05$, Tukey's pairwise comparison). The number of lateral shoots was significantly higher in treatment 2 compared to the treatment 4 and 5 (Fig. 2D, Tukey's pairwise comparison) while no difference was observed between other treatments. *C. vulgaris* tended to decrease in dry weight-length ratio with increasing temperature and brownification (Fig. 2E), although the change was not formally significant ($p < 0.060$).

Our mixed-model ANOVA showed significant main effects of treatments and time on the increment rate of total shoot length during the experiment (Fig. 3; Table S2) while interaction effects between time and treatments were not significant (Table S2). Shoot increment rates were significantly higher in July compared to May and June (One-way ANOVA, $F_{2, 33} = 6.76$, $p < 0.05$). On the other hand, total shoot increment rates were significantly higher for the treatment 2 compared to treatment 4 and 5 (Fig. 3).

5. Discussion

Environmental factors, such as light, nutrients and temperature, affect the growth and distribution of charophytes and other submerged macrophytes in shallow lakes (Joye and Rey-Boissezon, 2015; Hidding et al., 2010; Havens et al., 2004). Our results, aiming at assessing how predicted future increases in both temperature and brownification will affect charophytes, showed an increase in relative growth rate (RGR) of *C. vulgaris* at 100% increase in brownification and 2 °C increase in temperature compared to ambient conditions. Exposure of humic substances to UV radiation can degrade DOC and release CO_2 (Salonen and Vähätalo, 1994) that can be used by charophytes during photosynthesis. In addition, a previous study shows that DOC can be photochemically transformed into dissolved inorganic carbon (DIC- including bicarbonate) (Johannessen et al., 2007) and many submerged macrophytes, including charophytes, take advantage of bicarbonates for the photosynthetic process (Madsen and Sandjensen, 1991). Therefore, increased DOC due to brownification of freshwater may act as an additional resource for charophyte growth. The growth and distribution of *C. vulgaris* are generally found to be positively related to temperature increases (Puche et al., 2018; Joye and Rey-Boissezon, 2015). A previous study has shown a seven-fold increase in RGR of *C. vulgaris* when temperature was increased from 23 °C to 27 °C (Rojo et al., 2017). However, in our study a 4 °C increase in temperature along with 200% increase in brownification reduced the growth of *C. vulgaris* which was likely due to light limitation as brownification increased. Earlier studies have shown that light limitation is one of the most critical factors responsible for the decline of charophytes (Schubert et al., 2018; Blindow et al., 2014) and in shallow water ecosystems this decline occurs rapidly at a certain critical turbidity (Hidding et al., 2016; Scheffer et al., 1993). Therefore, beyond a certain threshold of brownification, the negative effect from light limitation on charophyte growth may no longer be compensated by increasing temperatures. Our experimental design provides crucial information regarding the change in charophyte growth in response to simultaneously elevated warming and brownification along a gradient. However, this experimental design does not allow for separation of the relative strength of each stressor. It is possible that there are complex interactions between the two stressors on the growth of charophytes, and that these effects are dependent on species-specific traits as well as local environmental conditions (Puche et al., 2018; Rojo et al., 2017; Joye and Rey-Boissezon, 2015).

We found that the total shoot length of *C. vulgaris* increased with temperature and brownification up to +2 °C and +100% brownification along the gradient. This is likely due to the morphological traits that

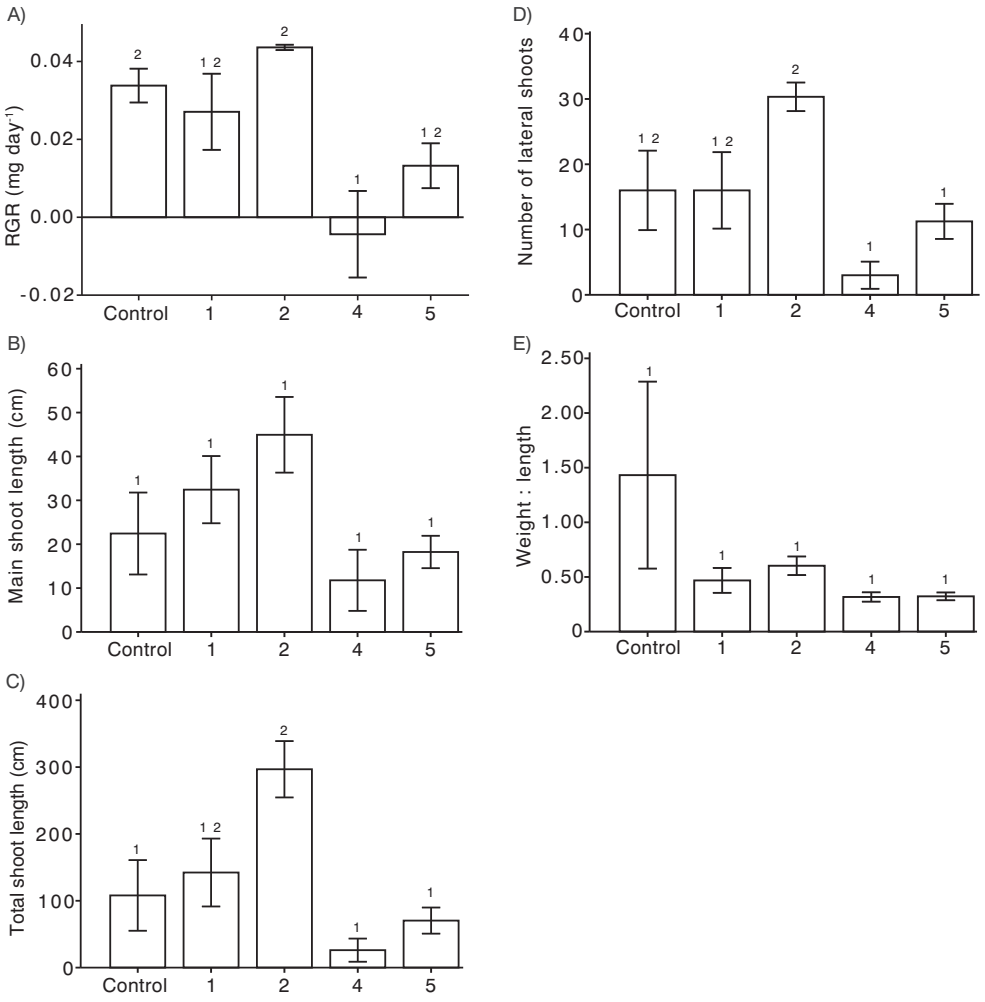

Fig. 2. Mean relative growth rate (RGR)(A), increment of main shoot length (B), increment of total shoot lengths (C), total number of lateral shoots (D) and ratio between macrophyte dry weight and length of *Chara vulgaris* at the end of the experiment (E), at different temperatures and brownification treatments (mean ± 1 SE). Similar letter above bars indicate no significant difference between the treatments (Oneway-ANOVA, Tuckey pairwise comparison) (Control = ambient temperature, no color added; 1 = +1 °C and 50% brownification; 2 = +2 °C and + 100% brownification, 4 = +4 °C and 200% brownification and 5 = +5 °C and 250% brownification).

allow charophytes and other submerged macrophytes to overcome low light conditions since stem elongation towards the water surface will help charophytes to retrieve light (Asaeda et al., 2007). Light limitation usually causes elongation of thalli in charophytes growing in deep waters, such as *Lychnothamnus barbatus* (Pelechaty et al., 2017), *Nitellopsis obtusa* (Larkin et al., 2018), and *Chara fibrosa* (Asaeda et al., 2007). However, we observed a decline in total shoot length of *C. vulgaris* in the treatments where brownification was elevated above +200% compared to the controls. Our study also showed a temporal variation in the rates of total shoot increment where highest rates were observed in July and at +2 °C and +100% brownification. We also found that the dry weight-length ratio of *C. vulgaris* eventually declined with increasing temperature and brownification, implying that *C. vulgaris* uses more energy for shoot elongation compared to biomass allocation at the higher end of the gradient of brownification and temperature increases. A similar trend was also observed for *Chara hispida* grown at different photosynthetically active radiations (PAR) where the weight-length ratio decreased with decreased PAR (Schneider et al., 2006).

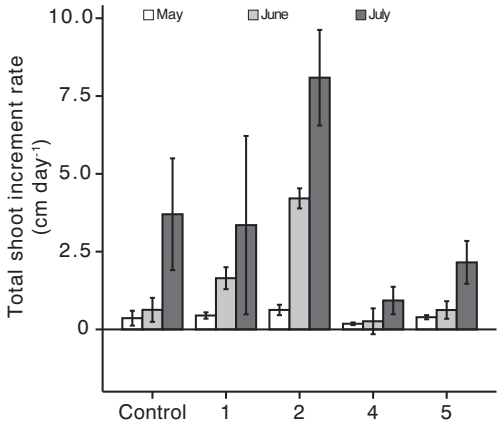

Fig. 3. Total shoot increment rates of *Chara vulgaris* at different temperatures and brownification treatments (mean ± 1 SE) (Control = ambient temperature, no color added; 1 = +1 °C and 50% brownification; 2 = +2 °C and + 100% brownification, 4 = +4 °C and 200% brownification and 5 = +5 °C and 250% brownification).

Mesocosm experiments provide a strong predictive basis to understand the response of organisms to ongoing environmental changes (Urrutia-Cordero et al., 2017; Stewart et al., 2013; Spivak et al., 2011). Our experiment captured the growth of charophytes under a scenario of climate warming and brownification conditions in north temperate lakes (Weyhenmeyer et al., 2016; IPCC, 2013). However, caution should be taken to scale up results to future climate change scenarios (Stewart et al., 2013). First, the temporal scales of projected natural increase in warming and brownification (Weyhenmeyer et al., 2016; IPCC, 2013) for the next decades will be longer than in our experiment. Second,

our manipulations have been done with water from one lake and the background level of brownification may show spatial variations across different regions, which then might determine the threshold level of brownification after which charophytes start to decline. Moreover, phenotypic plasticity of populations and the ecotypes can influence the response of charophytes to environmental changes (Puche et al., 2018; Rojo et al., 2015).

In conclusion, we here show that a likely climate scenario of increased warming and brownification in concert will initially lead to increased growth of *C. vulgaris* in northern Europe. However, as brownification increases further, *C. vulgaris* will decline after crossing certain threshold levels for light limitations, which may cause rapid changes in the macrophyte communities, especially charophytes. The understanding on how simultaneously occurring stressors, such as global warming and brownification, may induce threshold effects on key species is crucial for accurate decision making and management in preparing for changes in ecosystem function and services from freshwater systems.

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Appendix A. Supplementary information

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.01.177.

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